

Adverse Conditions Which Cause Lack of Flagella in *Escherichia coli*

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Wild-type *Escherichia coli* was not motile when grown in tryptone broth under the following adverse conditions: the presence of high temperature [J. Adler and B. Templeton, J. Gen. Microbiol. 46:175–184, 1967; R. B. Morrison and J. McCapra, Nature (London) 192:774–776, 1961; K. Ogiuti, Jpn. J. Exp. Med. 14:19–28, 1936], high concentrations of salts, high concentrations of carbohydrates, high concentrations of low-molecular-weight alcohols, or the pressure of gyrase inhibitors. Under all these conditions, growth was necessary for the loss of motility. This loss of motility was correlated with a reduction in the amount of cellular flagellin. We isolated and studied mutants that are resistant to suppression of motility by some of these conditions, because of the ability to synthesize flagella under these conditions. The mutations were mapped to 42 min, a region of the chromosome where many of the flagellar genes map. We also studied the effect of a preexisting *gyrA* mutation which allowed flagellar formation in the presence of nalidixate.

The formation of bacterial flagella is sensitive to environmental conditions. In *Escherichia coli*, flagellar synthesis is inhibited by catabolite repression caused by growth on D-glucose as the carbon source (4) and by growth at high temperatures (4, 20, 22).

In this study, we investigated the regulation of *E. coli* flagellar formation by environmental cues. We studied the effects of high concentrations of inorganic ions, high concentrations of carbohydrates, high temperature, high concentrations of alcohols, and the presence of gyrase inhibitors. The possibility that inhibition of flagellar formation may be a means of conserving energy under adverse conditions is discussed.

MATERIALS AND METHODS

Strains and genetic manipulations. All bacterial strains used were derivatives of *E. coli* K-12. Chemotactically wild-type strains AW607 (33) and AW405 (5) were previously described. RP3098 ($\Delta fliC\text{-}fliA$) (31) was used as a nonmotile strain. The motile nalidixate-resistant mutant AW933 (*gyrA*) was constructed from nonmotile parental strain YK3421 (*gyrA fliC::lacZ*) (13) by replacing the *fliC::lacZ* fusion gene with a wild-type *fliC* gene by P1 transduction (25). Strain AW940 was constructed by infection of motile wild-type strain W3110 (18) with a P1vir (19) phage lysate of strain CAG12156 (*uvrC::Tn10*) (28); this strain was used as a donor strain for cotransduction experiments. P1 transduction was performed by the method of Miller (19). For examining cotransduction frequency, about 100 transductants were scored for each transduction.

Media. Tryptone broth contained 1% Bacto Tryptone (Difco) and 0.5% NaCl. For tryptone swarm agar, 0.25% Bacto Agar (Difco) was included in the tryptone broth.

Growth and motility assays. Cells from tryptone broth overnight cultures were inoculated (1:100 dilution) into tryptone broth under conditions as indicated. Growth was at

35°C unless otherwise indicated. Doubling times were determined by measuring optical density at 590 nm (OD₅₉₀) in the range of 0.3 to 0.8.

For the swarming assay, bacteria were inoculated into the centers of petri plates containing 30 ml of tryptone swarm agar (1); various compounds were added as indicated. The plates were incubated at 35°C (except in some cases at 42°C as indicated) for times shown in footnote *a* of Table 3. The MIC for inhibition of swarming in the presence of a chemical was defined as the lowest concentration that completely inhibited swarming during a 24-h incubation. Most chemicals were tested at serial dilutions with 100 mM intervals. A 10 mM interval was used when the MIC of a salt was lower than 200 mM. Intervals of 10 and 100 μ M were used to test the MICs of nalidixate and novobiocin, respectively.

For examining the motility of free-swimming cells, bacteria at mid-exponential phase (OD₅₉₀ of 0.4 to 0.6) were used. The motility was observed by phase-contrast microscopy, and the cells were videotaped. The videotapes were then subjected to computer analysis, and the linear speed of the cells was determined according to the procedure of Sager et al. (23) with the following modification: cells having an average swimming speed of less than 5 μ m/s were not eliminated.

Immunoblotting for whole-cell flagellin. The amount of total flagellin (intracellular flagellin plus flagella) was determined by immunoblotting (12). Protein samples were prepared by boiling cells in Laemmli buffer (15) containing 0.1% β -mercaptoethanol. A total of 5 μ g of protein (determined by the bicinchoninic acid protein assay) (30) was loaded onto each lane of a sodium dodecyl sulfate–10% polyacrylamide gel. Flagellin was prepared as described by Edelmans and Gallant (9). The gels were run in a Bio-Rad mini-PROTEAN II dual-slab cell. Samples were transferred to nitrocellulose membranes by use of a Hoefer Transphor (TE50), probed with rabbit anti-flagellum antibody, and developed with goat anti-rabbit antibody conjugated with peroxidase by use of 4-chloro-1-naphthol and hydrogen peroxide.

Isolation of mutants. Chemotactically wild-type cells were mutagenized with nitrosoguanidine (as in the case of AW760) or with ethyl methanesulfonate (as in the case of AW780).

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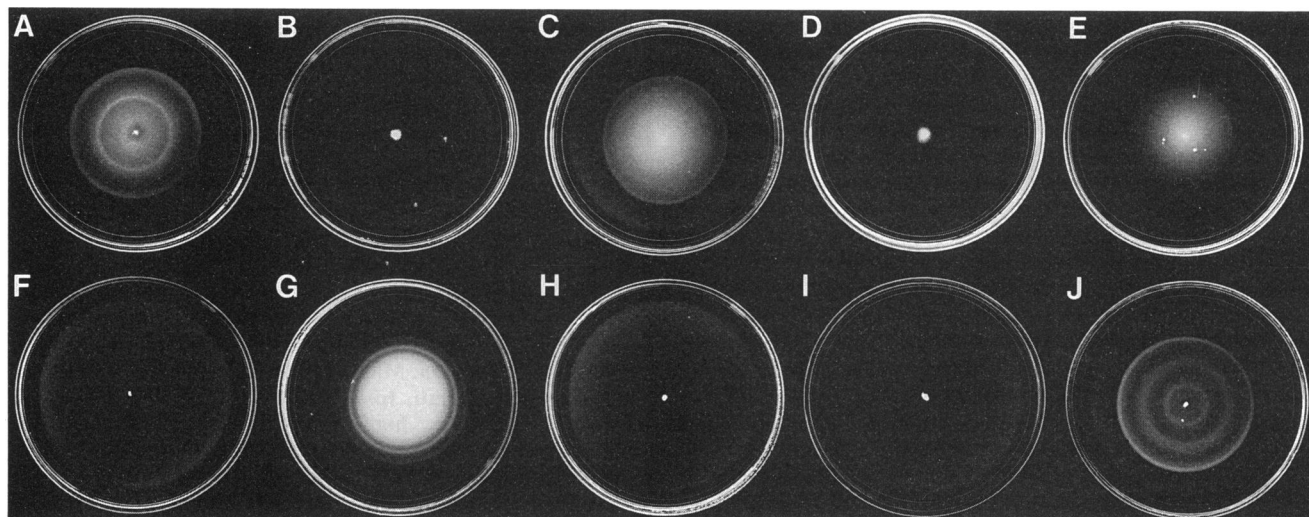


FIG. 1. The effects of adverse conditions on the swarming ability of wild-type and mutant strains in tryptone broth with the conditions described. AW607 is a wild-type strain, AW760 is a carbohydrate-resistant swarm mutant, AW771 is a salt-resistant swarm mutant, AW780 is a temperature-resistant swarm mutant, and AW933 is a nalidixate-resistant swarm mutant. (A) AW607; (B) AW607 in 500 mM added NaCl; (C) AW771 in 500 mM added NaCl; (D) AW607 in 600 mM sucrose; (E) AW760 in 600 mM sucrose; (F) AW607 at 42°C; (G) AW780 at 42°C; (H) AW607 in 700 mM ethanol; (I) AW607 in 20 μ M nalidixate; (J) AW933 in 20 μ M nalidixate. For those conditions under which swarming occurred, swarming was photographed when the diameter of the swarm had reached 5 cm. The incubation times for swarm plates were 10 h for panel A, 13 h for panels F and G, and 14 to 20 h for the remaining panels.

Mutagenesis was performed according to the protocol of Miller (19). Some mutants (e.g., AW771) were isolated without mutagenesis. Cells were inoculated into the centers of tryptone swarm-agar plates under conditions indicated in Results. The plates were incubated at 35°C (or at 42°C in the case of isolating temperature-resistant swarm mutants) for several days until swarms appeared. Mutants were picked from the edge of a swarm and purified as single-colony isolates.

RESULTS

Swarming ability of a wild-type strain. The ability of *E. coli* to swarm involves motility, chemotaxis, and growth (1). A typical swarm pattern for a chemotactically wild-type strain of *E. coli* (AW607) is shown in Fig. 1A. The inhibitory effect of adverse environmental conditions was examined by this swarm-plate method. The MIC was measured as described in Materials and Methods.

(i) **Effects of inorganic ions.** Swarming decreased when the ionic concentration was increased. The swarming of a wild-type strain (AW607) was completely inhibited by 500 mM NaCl in excess of the 86 mM normally present in tryptone broth (Fig. 1B; Table 1). The MICs of a variety of other salts for the wild-type strain were determined (Table 1). For chloride salts, cation effectiveness, from strongest to weakest, was $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{Ba}^{2+} > \text{Sr}^{2+} > \text{Cs}^+ > \text{Li}^+ > \text{Rb}^+ > \text{K}^+ = \text{Na}^+ = \text{NH}_4^+$. Similar results were obtained for nitrate salts. Divalent cations were more effective than monovalent cations. Anion effectiveness, from strongest to weakest, was $\text{F}^- > \text{I}^- > \text{Br}^- = \text{NO}_3^- = \text{SO}_4^{2-} > \text{Cl}^-$ for Na^+ and K^+ salts.

(ii) **Effects of carbohydrates.** The swarming of *E. coli* was completely inhibited by 600 mM sucrose and 600 mM ribitol (Fig. 1D; Table 2), both nonmetabolizable carbohydrates (2). D-Sorbitol (700 mM), a metabolizable carbohydrate (2), also completely inhibited swarming (Table 2).

(iii) **Effects of temperature.** The motility of *E. coli* is known to be inhibited by growth at 42°C (see the introduction). Consistent with this observation, AW607 did not swarm when incubated at 42°C (Fig. 1F; Table 2).

(iv) **Effects of low-molecular-weight alcohols.** The swarming of wild-type strain AW607 was completely inhibited by 700 mM (4%) ethanol (Fig. 1H; Table 2). Methanol, isopropanol, and *n*-propanol also inhibited motility, with MICs at 1.8 M (7%), 600 mM (3%), and 200 mM (1.5%), respectively (Table 2).

(v) **Effects of gyrase inhibitors.** The swarming of wild-type strain AW607 was completely inhibited by 20 μ M nalidixate (Fig. 1I; Table 2) or 200 μ M novobiocin (Table 2).

(vi) **Effects of high viscosity.** The effect of high viscosity on the motility of chemotactically wild-type strain AW405 grown in liquid medium was studied. The cells were grown in tryptone broth with 10% (wt/vol) polyvinylpyrrolidone (PVP-360) or 10% (wt/vol) Ficoll 70 or 10% (wt/vol) Ficoll 400 to an OD_{590} of 0.4 to 0.6, centrifuged out of the medium, and resuspended in tryptone broth without agents causing high viscosity. The cells were fully motile as judged by microscopic observation. Because conditions that inhibit motility were being studied, no further work with high viscosity was performed.

Similar effects of the conditions tried with strain AW607 were found with the wild-type strain AW405 (data not shown).

Isolation and characterization of mutants which swarm under adverse environmental conditions. Mutants swarming under adverse conditions were isolated as described in Materials and Methods. Eleven independent mutants that swarmed with 500 mM KCl present were isolated, and one of the mutants, AW771, was studied further. This mutant swarmed on tryptone plates containing 500 mM added NaCl (Fig. 1C) or 500 mM KCl. One independent mutant which swarmed with 500 mM added NaCl present was isolated. Two independent mutants whose swarming on tryptone

TABLE 1. Effects of salts in growth media on swarming ability, motility, and growth of wild-type strain AW607

Condition	MIC for swarming (mM) ^a	Motility after growth in condition indicated ^b	Motility in absence of growth ^c	Relative doubling time ^d
LiCl	300	—	+	0.9
NaCl ^e	500	—	+	1.5
KCl	500	—	+	1.5
RbCl	400	—	+	1.9
CsCl	200	—	+	2.2
NH ₄ Cl	500	—	+	1.3
LiNO ₃	150	—	+	0.8
NaNO ₃	300	—	+	2.0
KNO ₃	300	—	+	2.0
RbNO ₃	300	—	+	4.3
CsNO ₃	160	—	+	3.0
NH ₄ NO ₃	300	—	+	2.3
MgCl ₂	80	—	+/-	0.8
CaCl ₂	70	—	+/-	0.9
SrCl ₂	150	—	+/-	1.0
BaCl ₂	90	—	+/-	2.8
Mg (NO ₃) ₂	70	—	+/-	0.6
Ca (NO ₃) ₂	60	—	+/-	0.7
Sr (NO ₃) ₂	90	—	+/-	0.8
Ba (NO ₃) ₂	60	—	+/-	1.5
NaF	70	—	+	2.1
NaBr	300	—	+	1.1
NaI	200	—	+	1.0
KF	70	—	+	1.4
KBr	300	—	+	1.0
KI	190	—	+/-	2.2
Na ₂ SO ₄	300	—	+/-	0.9
K ₂ SO ₄	300	—	+	1.0
(NH ₄) ₂ SO ₄	300	—	+	1.3

^a The MIC for swarming was measured as described in Materials and Methods.

^b Cells were grown in tryptone broth to mid-exponential phase (OD₅₉₀ of 0.4 to 0.6) under the indicated conditions (at the MIC). The motility of free-swimming cells was judged by microscopic observation. —, more than 99% of the population was nonmotile.

^c The cells at mid-exponential phase were suspended in tryptone broth under the indicated conditions (at the MIC) plus 20 μ M chloramphenicol. Motility was then judged by microscopic observations as described in footnote *b* after a 5-h incubation. +, more than 90% of the population was motile; +/-, about 50% of the population was motile (see "Requirement of growth for loss of motility under adverse environmental conditions" in text).

^d The doubling time for the wild-type strain AW607 grown in tryptone broth under the indicated conditions was measured (see Materials and Methods); the relative doubling time is the ratio of the doubling time with the conditions present to the doubling time in tryptone broth alone.

^e For NaCl, the concentration is the amount above the 86 mM normally present in tryptone broth.

plates was resistant to 600 mM sucrose were isolated, one of these being AW760 (Fig. 1E). That mutant was also able to swarm on tryptone plates containing 600 mM ribitol or 700 mM D-sorbitol. Ten temperature-resistant (42°C) swarm mutants were isolated (7a), and one of them (AW780) was studied further (Fig. 1G). We have tested a nalidixate-resistant mutant, AW933 (*gyrA*) (25) and found that it was able to swarm (Fig. 1J) in the presence of up to 60 μ M nalidixate.

Motility of wild-type and mutant cells. To determine

TABLE 2. Effects of carbohydrates, temperature, alcohols, and gyrase inhibitors on the swarming ability, motility, and growth of wild-type strain AW607

Condition	MIC or minimum temp for swarming ^a	Motility ^b	Motility in absence of growth ^c	Relative doubling time ^d
Carbohydrates				
Ribitol	600 mM	—	+	1.2
D-Sorbitol	700 mM	—	+	1.2
Sucrose	600 mM	—	+	1.7
Temp	42°C	—	+	0.8
Alcohols				
Methanol	1.8 M	—	+	— ^e
Ethanol	700 mM	—	+	—
Isopropanol	600 mM	—	+	—
n-Propanol	200 mM	—	+	—
DNA gyrase inhibitors				
Nalidixate	20 μ M	—	+	1.8
Novobiocin	200 μ M	—	+	1.2

^a See footnote *a* of Table 1.

^b See footnote *b* of Table 1.

^c See footnote *c* of Table 1.

^d See footnote *d* of Table 1.

^e See "Rate of growth under adverse environmental conditions" in text.

whether adverse conditions affect the chemotaxis of *E. coli* without affecting motility directly, the motility of cells grown in liquid culture under each condition was examined microscopically. Wild-type AW607 was not motile when grown at the MIC for all the conditions (Tables 1 and 2). Thus, there appears to be a direct effect of the conditions on motility.

The dose-response effect of NaCl or KCl on motility of cells was measured by computer analysis (see Materials and Methods). The linear speed of wild-type cells was affected at 300 mM and higher added NaCl, but it was unaffected at lower concentrations (Fig. 2). Similar data were obtained by growing the cells with various concentrations of KCl (data not shown). Analysis of a nonmotile strain of *E. coli* yielded a linear speed of 3 μ m/s; this indicates the threshold for the lowest linear speed the system can analyze. Cells grown in 400 mM added NaCl showed a speed of 3 μ m/s and thus could be nonmotile. However, a direct visual observation of the cells indicated that they were motile, albeit slow, and wild-type cells are capable of swarming slowly at this concentration. Thus, computer analysis is not useful for measuring the speed of very slow cells. When grown at 500 mM added NaCl, the cells moved at a speed of 3 μ m/s and were not visibly motile.

In contrast to the wild-type strain, the salt-resistant swarm mutant (AW771) was motile after growth in up to 600 mM added NaCl (Fig. 2) or 600 mM KCl (data not shown). The linear speed of mutant AW771 was higher than that of wild-type strain AW607 even with no salt present and was not affected by concentrations below 600 mM added NaCl (Fig. 2) or 600 mM KCl. At 600 mM added NaCl, the linear speed of the mutant was reduced (Fig. 2), as it was at 600 mM KCl (data not shown).

Judged microscopically, a carbohydrate-resistant swarm mutant (AW760), a temperature-resistant swarm mutant (AW780), and a nalidixate-resistant swarm mutant (AW933) were motile after growth in up to 800 mM sucrose, at 42°C, and in up to 60 μ M nalidixate, respectively. The results from

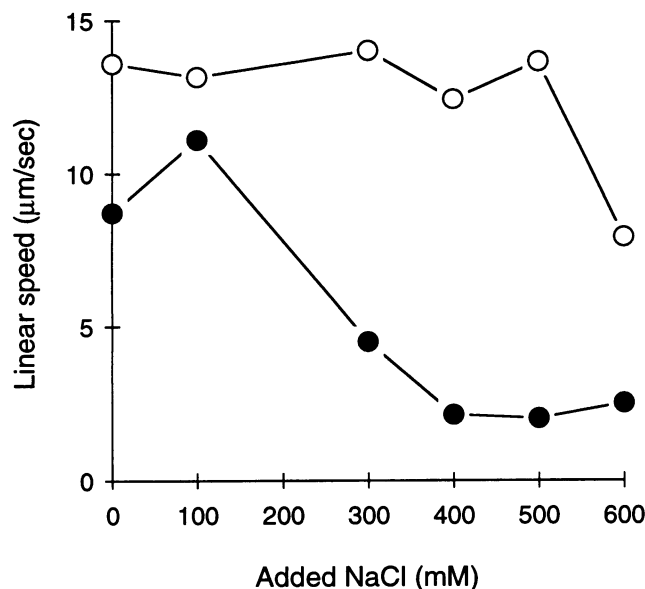


FIG. 2. Effects of growth in tryptone broth with added NaCl on motility of wild-type and mutant strains. The linear speeds of the wild-type strain (AW607) and the salt-resistant swarm mutant (AW771) were measured as described in Materials and Methods. The concentrations of NaCl indicated were the additional NaCl in excess of the 86 mM normally present in tryptone broth (see Results). A nonflagellated mutant (RP3098) showed a linear speed of 3 μ m/s. ●, AW607; ○, AW771.

growth in liquid culture are thus consistent with the swarm-plate results.

Rate of growth under adverse environmental conditions. The rate of growth of wild-type strain AW607 was decreased under most conditions that inhibit motility, including most inorganic salts, carbohydrates, alcohols, or gyrase inhibitors, but the growth was not completely blocked (last column of Tables 1 and 2). The presence of alcohols, in addition to decreasing the growth rate, caused the cells to reach stationary phase at an OD_{590} of 0.4 to 0.6 (compared with a final OD_{590} of about 3.0 for cells grown in tryptone broth); this made it difficult to calculate an accurate growth rate.

Some conditions, on the other hand, actually slightly increased the rate of growth of the wild-type strain compared with the growth rate of the strain at 35°C in tryptone broth alone (Table 1). These conditions included the presence of the divalent cation calcium, magnesium, or strontium. Lithium salts and 42°C also increased the growth rate (Tables 1 and 2). Several conditions, such as $SrCl_2$, NaI, KBr, and K_2SO_4 , did not substantially affect the growth rate (Table 1). This shows that there is not a simple relationship between growth rate and loss of motility in wild-type cells.

The salt-resistant swarm mutant (AW771) was inhibited in its growth 1.5-fold by addition of 500 mM KCl or 500 mM added NaCl (compared with 1.5-fold for its parent), the carbohydrate-resistant swarm mutant (AW760) was inhibited 1.6-fold by addition of 600 mM sucrose (compared with 1.7-fold for its parent), the temperature-resistant swarm mutant (AW780) was stimulated 1.3-fold by growth at 42°C instead of 35°C (compared with 1.3-fold for its parent), and growth of the nalidixate-resistant swarm mutant (AW933) was not affected by 20 μ M nalidixate (compared with an

inhibition of 1.8-fold for its parent). Thus, the mutations affect the loss of motility of the cells caused by the conditions without altering the growth rate changes caused by the conditions, with the exception of the *nalA* mutation.

Requirement of growth for loss of motility under adverse environmental conditions. The wild-type cells were observed in media with the adverse conditions plus a protein synthesis inhibitor (20 μ M chloramphenicol). None of those conditions could completely inhibit motility even after a 5-h incubation (Tables 1 and 2, third column). Some salts, such as the divalent cation salts, as well as KI and Na_2SO_4 , inhibited motility (Table 1). However, clumping of the cells was also observed under these conditions, and that appears to be the cause of the inhibition of motility; in contrast to the clumped cells, individual free-swimming cells were motile. Thus, growth under adverse conditions is necessary for loss of motility.

Amount of flagellin. An immunoblot was performed to analyze the level of flagellin (14), the major flagellar protein. Whole-cell total flagellin (intracellular plus extracellular) was measured in wild-type and mutant strains grown under adverse conditions. Figure 3 shows that the amount of total flagellin was reduced when the wild-type strain (AW607) was grown in 500 mM added NaCl, 600 mM sucrose, 700 mM ethanol, 20 μ M nalidixate, or at 42°C. A similar reduction of total flagellin was detected when wild-type cells were grown in 200 μ M novobiocin, 500 mM KCl, 100 mM KF, 60 mM Ca (NO_3)₂, or 70 mM Mg (NO_3)₂ (data not shown).

In contrast, an amount of whole-cell total flagellin similar to the levels found in motile wild-type cells was detected in the salt-resistant swarm mutant (AW771) that was grown in 500 mM added NaCl, in the carbohydrate-resistant swarm mutant (AW760) that was grown in 600 mM sucrose, in the temperature-resistant swarm mutant (AW780) that was grown at 42°C, and in the nalidixate-resistant swarm mutant (AW933) that was grown at 20 μ M nalidixate (Fig. 3).

Mutants which are motile under adverse conditions. Each of the mutants selected for swarming under one condition (see "Isolation and characterization of mutants which swarm under adverse environmental conditions" above) was tested under all the other conditions (Table 3). The nalidixate-resistant (*gyrA*) mutant AW933 showed the ability to swarm under the fewest conditions, being capable of swarming only in nalidixate (Table 3). On the other hand, the other three mutants showed broader resistances. The temperature-resistant swarm mutant was able to swarm in the presence of sucrose, ethanol, and novobiocin in addition to high temperature (Table 3). The sucrose-resistant swarm mutant was able to swarm in ethanol as well as on sucrose, and the salt-resistant swarm mutant was able to swarm in the presence of sucrose, high temperature, and ethanol in addition to high salt (Table 3). All three latter mutants were resistant to a mixture of 25 mM D-glucose, 25 mM D-gluconate, and 25 mM D-mannitol, sugars that cause catabolite repression (26) (Table 3).

Inhibitory effects on motility are additive. We studied the effects of combinations of conditions on the motility of AW405. The bacteria were grown in liquid culture, and the motility was judged microscopically. Temperature and salt acted additively. Growth at 35°C with 500 mM KCl eliminated motility, but growth at 30°C, which gave better motility than growth at 35°C, in the presence of 500 mM KCl, although severely inhibiting motility, did not abolish it; 650 mM KCl was required to completely abolish motility at 30°C. Similarly, 700 mM ethanol was required to suppress the motility of cells grown at 35°C, whereas only 500 mM

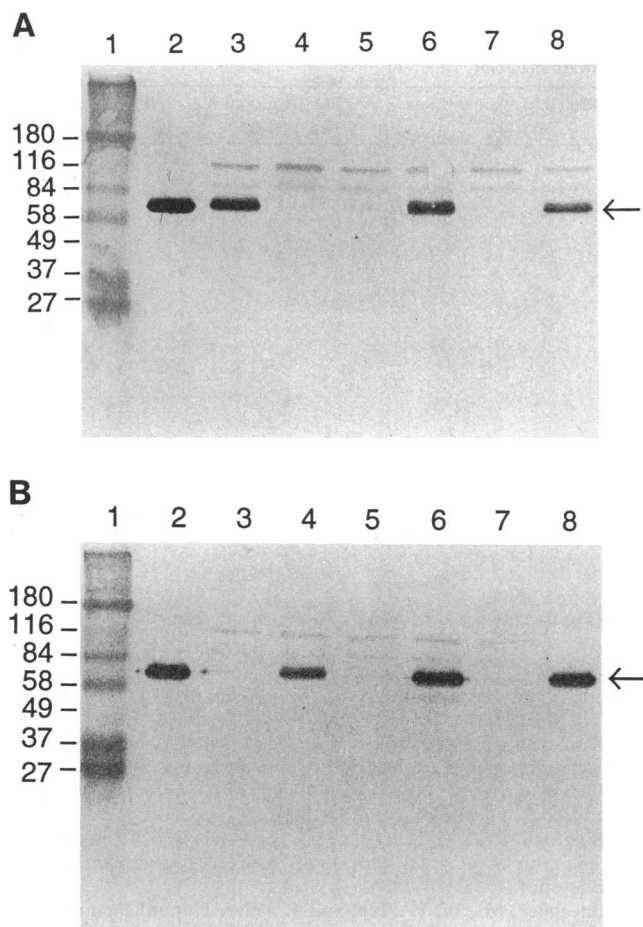


FIG. 3. The effects of adverse conditions on the amount of flagellin in wild-type and mutant strains. The immunoblotting method was used to assay whole-cell (i.e., intracellular plus extracellular) flagellin (see Materials and Methods). Approximately 5 µg of protein was loaded in each lane, except in lanes 2 and 8 of panel B and lane 2 of panel A, for which 0.5 µg of purified flagellin was used. The arrows point to flagellin. Molecular size markers (in kilodaltons) are indicated at the left. AW607 is a wild-type strain, AW771 is a salt-resistant swarm mutant, AW760 is a carbohydrate-resistant swarm mutant, AW780 is a temperature-resistant swarm mutant, and RP3098 is a nonmotile mutant that does not produce flagella. Wild-type and mutant strains were grown in tryptone broth with the conditions described as follows. (A) Lane 1, molecular size markers; lane 2, flagellin; lane 3, AW607; lane 4, RP3098; lane 5, AW607 at 42°C; lane 6, AW780 at 42°C; lane 7, AW607 in 500 mM added NaCl; lane 8, AW771 in 500 mM added NaCl. (B) Lane 1, molecular size markers; lane 2, flagellin; lane 3, AW607 in 600 mM sucrose; lane 4, AW760 in 600 mM sucrose; lane 5, AW607 in 20 µM nalidixate; lane 6, AW933 in 20 µM nalidixate; lane 7, AW607 in 700 mM ethanol; lane 8, flagellin.

ethanol was required to completely abolish the motility of cells grown at 38°C; cells grown at 38°C were less motile than cells grown at 35°C.

Similarly, the salt-resistant swarm mutant (AW771) could swarm at 42°C or at 35°C with 500 mM KCl or NaCl present, but it could not swarm with 500 mM KCl or NaCl present when grown at 42°C.

Preliminary genetic mapping of mutants. The mutations present in the salt-resistant swarm mutant (AW771), the carbohydrate-resistant swarm mutant (AW760), and the tem-

perature-resistant swarm mutant (AW780) had a 56 to 64% cotransduction frequency with *uvrC279::Tn10* (located at 42 min on the chromosome linkage map in the midst of a cluster of flagellar chemotaxis genes [6]).

DISCUSSION

This study describes a number of growth conditions which lead to loss of motility as demonstrated by swarming ability and microscopic examination. These conditions include growth in the presence of high concentrations of inorganic salts, high concentrations of carbohydrates, high concentrations of alcohols, high temperature, or the presence of gyrase inhibitors.

The flagellar chemotaxis regulon in *E. coli* contains over 40 genes that are organized in a regulatory hierarchy such that expression of the genes at the top of the hierarchy is required for expression of genes lower in the hierarchy (see review in reference 17). A large amount of energy is required to synthesize flagella. The amount of flagellin alone composes about 8% of the total cell protein when the flagellar operons are expressed optimally (21). The ability of the bacteria to shut down this costly process under adverse conditions might help them to survive.

It is interesting to note that for *E. coli* many of these conditions have been shown to be repellents at millimolar concentrations. This bacterium avoids high concentrations of inorganic salts and high osmolarity (3, 16). Alcohols and nalidixate are also known repellents (33). Thus, motile bacteria first carry out tactic responses away from these conditions. If that fails, they shut down their chemotaxis and flagellar-protein synthesis, which allows more energy for survival. It would be interesting to see whether other stresses such as carbon starvation, oxidative stress (10), and pH stress (7, 29) also affect *E. coli* flagellation.

We have demonstrated that motility is inhibited by growth in inorganic salts (Table 1). While carbohydrates cause loss of motility at about 600 to 700 mosM (Table 2), inorganic salts have a broader range of effect. Salts such as NaCl or KCl act at 500 mM, but the divalent ions have a stronger effect than would be expected just from considerations of osmolarity (Table 1). These ions, with the exception of barium, also do not reduce the growth rate as other salts (such as NaCl or KCl) or carbohydrates do. It is possible that they may not act by the same mechanism as salts, such as NaCl or KCl, or carbohydrates do.

Neither salts nor carbohydrates act by changing the viscosity of the medium; agents that confer either high macroviscosity alone or high microviscosity and macroviscosity together do not have a noticeable effect on the motility of cells.

Genetic studies will be useful in understanding the mechanism of nonflagellation resulting from adverse conditions. We present evidence for two types of mutations which can make cells motile under those conditions. Type I mutations are those which do not map in the flagellar region. An example is the mutant AW933 (25), which is motile in 60 µM nalidixate because of a *gyrA* mutation. It is known that the *gyrA* mutation will abolish the target site of nalidixate in the gyrase molecule and make cells no longer sensitive to it (8, 11, 24, 32). Type II mutations are those mutations which map in the flagellar region. *E. coli* mutants whose motilities are resistant to catabolite repression or high temperature have been reported (27). Mutations causing resistance to catabolite repression mapped at *flhD*; those causing resistance to high temperature mapped at *flhD* or *fliA* (27). Type

TABLE 3. Effects of adverse conditions on swarming ability of mutants

Condition ^a	Swarming ability of strain ^b :				
	AW607 (wild type)	AW771 (salt-resistant swarmer)	AW760 (sucrose-resistant swarmer)	AW780 (temperature-resistant swarmer)	AW933 (nalidixate-resistant swarmer)
35°C, tryptone broth	+	+	+	+	+
35°C, tryptone broth plus 500 mM KCl or added NaCl ^c	—	+	—	—	—
35°C, tryptone broth plus 600 mM sucrose	—	+	+	+	—
42°C, tryptone broth	—	+	—	+	—
35°C, tryptone broth plus 700 mM ethanol	—	+	+	+	—
35°C, tryptone broth plus 20 µM nalidixate	—	—	—	—	+
35°C, tryptone broth plus 200 µM novobiocin	—	—	—	+	—
35°C, tryptone broth plus D-glucose-D-gluconate-D-mannitol (25 mM each) ^d	—	+	+	+	—

^a For the first row of the table (35°C, tryptone broth), the swarm plates were incubated for 10 h; for the fourth row (42°C, tryptone broth), the plates were incubated for 13 h; for the remaining rows, plates were incubated for 24 h.

^b +, swarming observed; —, swarming not observed.

^c For NaCl, the concentration is the amount above the 86 mM normally present in tryptone broth.

^d This medium, which causes catabolite repression and thus inhibition of the synthesis of flagella, was described by Silverman and Simon (26).

II is also represented by the mutations reported in this study: the salt-resistant swarmer (AW771), the sucrose-resistant swarmer (AW760), and the temperature-resistant swarmer (AW780). All those mutations mapped near 42 min of the chromosome, where most of the flagellar genes are clustered. Type II mutations might affect flagellar genes by enhancing promoter strength or by causing a loss of a binding site for a repressional signal generated by adverse conditions.

The mechanism(s) of this regulation of motility is still unknown. The loss of motility under adverse conditions was blocked by a protein synthesis inhibitor (Tables 1 and 2); this suggests that gene regulation is involved. The loss of motility was correlated with a reduction in the amount of cellular flagellin (Fig. 3). Elsewhere, we report that the transcription of the motility-chemotaxis regulon and the synthesis of flagellar protein were indeed reduced under those conditions, and this process does not result from catabolite repression (25). However, the signals that regulate the expression of the flagellar genes under those adverse conditions remain unidentified.

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